Original Article

A Comparative Study On Liver Toxicity Profile Of Diclofenac Sodium and Piroxicam on Rabbits

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ABSTRACT

Objective: Aim of this study was to determine the clinical hepatotoxicity of diclofenac sodium and of piroxicam, and to evaluate whether these drugs could elicit liver cell destruction and anemia, and which drug is comparatively safer for prolong use.

Place and Duration of Study: This study was conducted in the department of Pharmacology, Faculty of pharmacy, University of Karachi, Karachi. Duration of study was 30 days.

Materials and Methods: Male 40 rabbits were equally divided into 4 groups, group A was served as control and the group B & C was diclofenac sodium (0.8mg/kg/day and 1.5mg/kg/day), and group D was of piroxicam (0.31 mg/kg/day) treated. All the animals were caged in pair in an iron caged with free access to grass and hay of standard diet and tap water for a period of 30 days. Diclofenac sodium in 2 different doses 0.8mg/kg/day, 1.5mg/kg/day and similarly Piroxicam (0.31mg/kg/day) dissolved in drinking water and was given orally for a period of 30 days. Control rabbits were given tap water. At the end of 30 days blood was collected through cardiac puncture from each rabbit and was analyzed to determine the levels of SGOT, SGPT, Bilirubin, ESR and Erythrocyte count.

Result: It was found that these drugs can induce severe hepatic damage but the ratio of liver toxicity is different, as evident by the elevation of serum aminotransferases, bilirubin and changes in hematological profile. The experimental results suggest that SGOT and SGPT levels were significantly increased in diclofenac sodium treated rabbits after 10 and 30 days ($P < 0.01$), while piroxicam treated rabbits showed significant result, ($P < 0.05$) only after 30 days of treatment.

The level of bilirubin was significantly increased in diclofenac sodium treated rabbits after 10 days and 30 days ($P < 0.01$) and piroxicam also showed significant result ($P < 0.05$) after 30 days treatment. Erythrocyte count decreased in both control and treated rabbits after 10 days but control results are not significant. After 30 days diclofenac sodium showed highly significant decrease in count of erythrocytes ($P < 0.01$), but piroxicam showed less significant results ($P < 0.05$). E.S.R values significantly increased in diclofenac sodium and piroxicam treated rabbits after 10 days and 30 days.

Conclusion: It can be concluded that diclofenac sodium and piroxicam both can play a role in inducing hepatocelllualr damage, but a greater increase in liver toxicity was seen in diclofenac sodium treated rabbits rather than piroxicam treated rabbits.

Key words: Diclofenac Sodium, Piroxicam, Hepatotoxicity, serum aminotransferases, Bilirubin.

INTRODUCTION

Pain is an unpleasant sensory and emotional experience, and one of the greatest services is to acquire skill in the management of pain. Analgesics are drugs that relieve pain and among them non-narcotic analgesics are those which act peripherally to achieve this effect[1]. When a tissue is injured or stimulated, there is increased synthesis of prostaglandins which act as mediators of inflammation, and a drug that prevents the synthesis of these is likely to be affective in relieving pain[2]. Non-steroidal anti-inflammatory drugs (NSAID's) are among the most widely used medications in the world because of their demonstrated efficacy in reducing pain and inflammation[3]. Their efficacy has been documented in a number of clinical disorders, including osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout, dysmenorrhea, dental pain and headache[4,5]. The basic mode of action is inhibition of the pro-inflammatory enzyme cyclooxygenase (COX). NSAID's as a class comprise both traditional nonselective NSAIDs, which nonspecifically inhibit both COX-1 and COX-2, and selective COX-2 inhibitors. Although effective for relieving pain and inflammation, NSAID's are associated with a significant risk of serious gastrointestinal adverse events with chronic use[6]. The use of analgesics has increased considerably and had led to the knowledge of some serious unwanted hepatotoxic effects of some commonly used NSAID's. Present study is designed to investigate the effects of Diclofenac sodium and piroxicam when administered for different duration and with different dosages[7].

Diclofenac and piroxicam are relatively nonselective cyclooxygenase inhibitors, have been widely used to treat inflammatory or nociceptive disorders such as rheumatoid arthritis, osteoarthritis, cervicobrachial syndrome, frozen shoulder and post operative pains[8] . Whereas these NSAID's when used in long term chronic diseases can cause liver damage with elevation of liver enzymes[9].

An elevation of ALT or AST was observed in patients receiving diclofenac when compared to other NSAIDs. Transaminase elevations were seen more frequently in patients with osteoarthritis than in those with rheumatoid arthritis. In addition to enzyme elevations seen in clinical trials, post marketing surveillance has found rare cases of severe hepatic reactions, including liver necrosis, jaundice, and fulminant fatal hepatitis with or without jaundice. Some of these rare reported cases underwent liver transplantation[10]. Diclofenac was found to generate protein adducts in the livers of treated mice as well as in rat hepatocytes via protein acylation by the drug glucuronide**.** In vitro experiments with cultured rat hepatocytes have shown, however, that the covalent binding of diclofenac is neither the only nor the major cause of acute cytotoxicity**.** Moreover, it is also suggested that diclofenac is more cytotoxic to rat hepatocytes than piroxicam due to cytochrome P-450 (CYP)-mediated metabolism**,** by the formation of reactive metabolite(s) by drug oxidation, which could be related to drug toxicity, has been reported. While piroxicam can cause liver damage in high doses by an immunoallergic mechanism & formation of ductopenia, but factors responsible for this chronic evolution are still unknown[11,12].

The objective of the present study is to determine the role of diclofenac sodium & piroxicam in inducing hepatocellulr damage, further to evaluate whether these drugs could elicit liver cell destruction and anemia, and which drug is comparatively safer for prolong use.

MATERIALS AND METHODS

- Locally bred 40 male rabbits weighing range 1.03 to 1.7kg were used for the experiment. They were caged in pair in an iron caged with free access to grass and hay of standard diet and tap water. Food intake was monitored weekly by giving rabbit's weighed amount of food and weighing the remaining food in the iron cage. Body weight, food intake, water intake, skin color and posture of all rabbits were monitored in pre-experimental period.

Drug Administration

Diclofenac sodium in 2 different doses 0.8mg/kg/day, 1.5mg/kg/day and similarly Piroxicam 0.31mg/kg/day dissolved in drinking water and was given orally. Control rabbits were given tap water. In the beginning of the experiment 40 rabbits were divided in to 4 groups, and labeled as:

1. Water treated (control).
2. Diclofenac sodium 0.8mg/kg/day treated.
3. Diclofenac sodium 1.5mg/kg/day treated.
4. Piroxicam 0.31mg/kg/day treated.

Blood was collected through cardiac puncture from each control rabbit in sodium citrate containing test tubes. Centrifugation method was used to obtain plasma. Plasma, samples were stored at 2-8^{o}C for the estimation of SGOT, SGPT, Bilirubin, ESR and Erythrocyte count.

The dosing was started from day 1 till day 30th. At 10th day after the dosing, body weight, food intake, water intake, behavioral monitoring and blood samples were collected in 3.8% sodium citrate containing test tubes by cardiac puncture. Centrifugation gave plasma, which was used for the different tests.

On 30th day of the dosing, body weight, food intake, water intake and behavioral monitoring, rabbits were sacrificed and blood was collected in 30 different 3.8% sodium citrate (anti-coagulant) containing test tubes. Blood was centrifuged and plasma was collected to perform the tests.

After separation of serum, liver enzymes SGOT, SGPT & Bilirubin were estimated by Spectrophotometer by using standard kit method. E.S.R is estimated by Westergen's tube method & RBC's count by Haug method.

Statistical analysis

Comparison of difference of mean between diclofenac sodium in two different doses, control group & piroxicam was made by using student's t-test. Rabbits liver enzymes like SGPT, SGOT and Bilirubin and blood parameters like E.S.R and Erythrocyte count, after 10day and 30day were statistically analyzed by two way ANOVA using a software "Minitab-15". A p value less than 0.05 were considered statistically significant and p value less than 0.005 were considered highly significant.

RESULTS

Figure No. 1: Shows the effect of diclofenac sodium at the dose of 0.8 and 1.5 mg/Kg/day and 0.31mg/kg/day piroxicam on rabbit liver enzyme SGOT and SGPT (U/I). Data analyzed by two-way ANOVA (df = 1, 36), shows that SGOT and SGPT were significantly increased ($P<0.05$) in diclofenac sodium 0.8 and 1.5mg/Kg/day treated rabbits after 10 days and highly significant ($P<0.01$) results obtained in rabbits after 30 days. But piroxicam 0.31mg/Kg/day treated rabbits show ($P<0.01$) significant effects only after 30 days of treatment.

Figure No.2: Show the effect of diclofenac sodium and piroxicam on rabbit Erythrocyte count & ESR. Fig 2a

Shows effect of o.8mg/Kg/day, 1.5mg/kg diclofenac Na & 0.31mg/kg piroxicamon rabbit Erythrocyte count after10 and 30 days. Data analyzed by two - way ANOVA (df = 1, 36), shows that erythrocyte count significantly increases (P<0.05) in diclofenac sodium 0.8 mg/Kg/day treated rabbits after 10 days and highly significant (P<0.01) results obtained in rabbits after 30 days, while piroxicam show less significant results after 10 days but significant results (P<0.05) after 30 days treatment.

And fig 2b shows effect of o.8mg/Kg/day, 1.5mg/kg diclofenac Na & 0.31mg/kg piroxicam on rabbit ESR after10 and 30 days. That also show highly significant results (P<0.05) after taking both diclofenac sodium and piroxicam for10 days, and highly significant results (P<0.01) obtained after 30 days.

Figure No. 1: Effect of Diclofenac Sodium 0.8mg/Kg 1.5mg/Kg & Piroxicam 0.31mg/Kg on Rabbit Liver Enzyme Sgot & Sgpt (U/I)

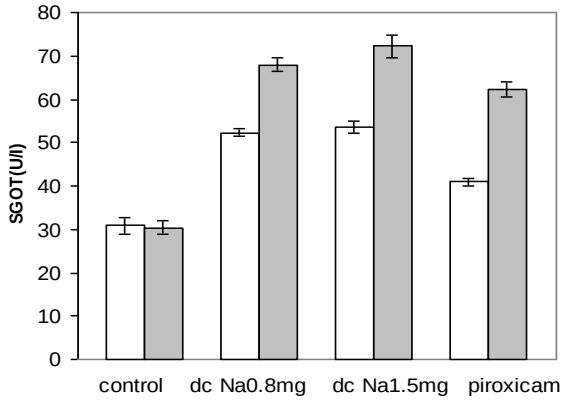

Figure No.1a

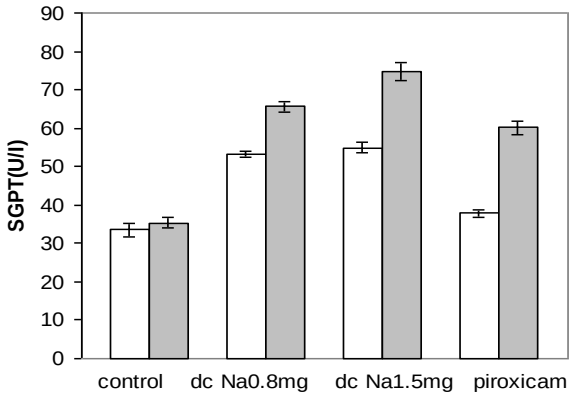

Figure No.1b

Figure 1: Effect of diclofenac sodium & piroxicam on rabbit liver enzymes SGOT & SGPT.

Figure 1a: Shows effect of o.8mg/Kg/day diclofenac Na, 1.5mg/Kg/day diclofenac Na & piroxicam on rabbit liver SGOT after10 and 30 days, while Figure 1b: Shows effect of diclofenac sodium 0.8mg and 1.5mg/kg/day and piroxicam 0.31mg/kg/day on rabbit liver SGPT after10 and 30 days,

Figure No.2: Effect of Diclofenac Sodium 0.8mg/Kg , 1.5mg/Kg & Piroxicam 0.31mg/Kg on Rabbit Erythrocyte Count /Mm^3& Esr(Mm)

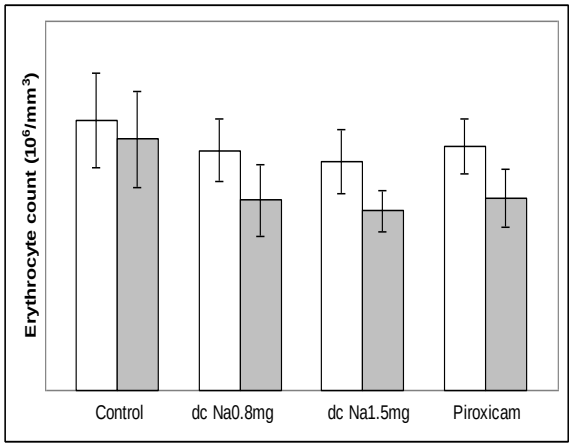

Figure No. 2a

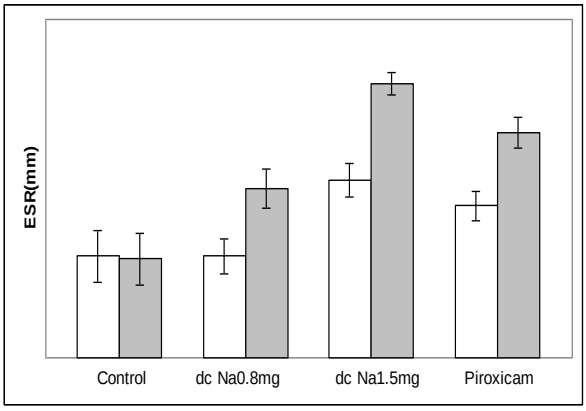

Figure No. 2b

Figure No. 2: Effect of diclofenac sodium and piroxicam on rabbit Erythrocyte count & ESR.

Figure No. 2a Shows effect of o.8mg & 1.5mg/kg/day diclofenac Na and 0.31mg/kg/day piroxicam on rabbit Erythrocyte count after10 and 30 days.

Figure No. shows effect of 0.8mg & 1.5mg/Kg/day diclofenac Na and 0.3mg/kg/day piroxicam on rabbit ESR count after 10 and 30 days.

Table No.1: Show the effect of diclofenac sodium at the dose of 0.8 and 1.5 mg/Kg/day and 0.31mg/kg piroxicam on rabbit Bilirubin (mg/dl).). Data analyzed by two-way ANOVA (df = 1, 36), shows that Bilirubin significantly increases (P<0.05) in diclofenac sodium 0.8 and 1.5mg/Kg/day treated rabbits after 10 days and highly significant (P<0.01) results obtained in rabbits after 30 days. Piroxicam 0.31mg/kg/day treated rabbits show (P>0.05) insignificant effects after 10 days but highly significant effect (P<0.01) after 30 days treatment.

Table No.1: Effect of Diclofenac Sodium 0.8mg/Kg , 1.5mg/Kg and Piroxicam 0.3mg/Kg on Rabbit Bilirubin (Mg/Dl)

	on Day 10	on Day 30	two-way ANOVA (df = 1, 36)
Control	0.4059 ± 0.129	0.478 ± 0.111	F-Interaction= 4.15, P <0.05
Diclofenac Na 0.8mg/Kg/day	0.5496* ± 0.094	0.761**± 0.09	
Diclofenac Na1.5mg/Kg/day	0.913**± 0.16	1.147**± 0.162	
Piroxicam 0.31mg/Kg/day	0.5729*± 0.035	0.93**± 0.061	

Values are mean ± S.D. (n=10). Significant differences by Newman-Keuls test *p<0.05 is significant & **p<0.01 is highly significant, as compared to control rabbits, following data analyzed by Two Way ANOVA df (1,36).

DISCUSSION

The drugs that are used as analgesic and anti-inflammatory agents are usually used as over the counter drugs. This implies to a fact that there is no check as to whether the patient is using rationally or irrationally these drugs in different inflammatory conditions.

Diclofenac sodium & piroxicam are n**on**-steroidal anti-inflammatory drugs (NSAIDs) used for most rheumatic disorders, and are used in large numbers as anti-inflammatory and analgesics, both as prescripti**on** drugs and over the counter purchases. The epidemiological risk of clinically apparent liver injury is greater (200 cases per 100 000 patient years) from diclofenac sodium than piroxicam (1–8 cases per 100 000 patient years), but when it occurs, it can be serious and can cause diagnostic c**on**fusi**on**. The adverse effect profile needs to be studied [13,14]

Diclofenac can impair ATP synthesis by mitochondrion which is in accordance to our result indicating that they can cause hepatotoxicity over long period of administration of these drugs. When we administered diclofenac in the dose of o.8mg/kg and 1.5mg/kg both profile show that the level of SGOT and SGPT were significantly elevated. The toxicity may be related to the impairment of ATP synthesis and also by impairing NADPH which are required to reduce the toxicity of hepatocytes.

This toxicity is also related to a fact that diclofenac sodium can form a toxic metabolite, and can also cause binding of drug to hepatic proteins[15].

The toxic metabolite formed is 4'hydroxy diclofenac by the action of CYP2CP[16].The results also showed that the toxicity profile of diclofenac sodium and potassium changed when the duration of therapy was increased. So the levels of SGOT and SGPT were further increased significantly after the period of 30 days dosing, thus indicating that the hepatotoxicity is not only dose dependent but is also duration dependent.

The changes in the liver enzymes i.e. transaminases were not only significant but also the level of bilirubin was found to be elevated after the administration of these drugs. This is also in accordance with results reported earlier[17] where jaundice was presented in a patient treated with diclofenac. The level of bilirubin increased indicates that the hepatotoxicity may be progressed towards liver necrosis. The toxic effects of diclofenac and its metabolites, along with hypersensitivity reactions may be the suggested molecular mechanism of liver injury.

The reason of marked elevated transaminases in the rabbits liver may be attributed to the fact that the metabolic pathways of diclofenac results in the formation of a metabolite that leads to acute lethal cell injury.

The increased level of bilirubin may also leads to certain renal dysfunctions as increased clearance and precipitation of bilirubin could lead to the renal nephritic syndromes. This finding may also be related previous study[18], who has reported that there may be renal complications due to the use of NSAID's particularly diclofenac partially due to the development of secondary membranous nephropathy. This was also supported by the study that the renal complications were reversed after the withdrawal of diclofenac and showed response if treatment with prednisolone was initiated.

The significant rise in the level of bilirubin could also be attributed to the findings that the total erythrocyte count and Hb was significantly reduced after the administration of diclofenac. The increased hemolysis of the R.B.C's can also lead to the increased level of bilirubin which could further be exaggerated by the liver toxicity, as liver could not decrease the concentration of bilirubin of serum through the clearance mechanism. As reported[19] that there may be revised forms of hepatic injury induced by diclofenac. In this type of injury there is a combined failure of canalicular pumps and other intracellular processes also that allow toxic bile acids to accumulate, causing secondary injury to hepatocytes. There may be also the likelihood to develop the injuries to the cells of the bile duct. The injury to the hepatocyte may occur due to the disruption of the intra-cellular calcium homeostasis that leads to the disassembly of acute fibrils at the surface of hepatocyte. This may result in blabbing of the cell membrane rupture, and cell lysis.

The other possible mechanism may involve the combination of the drug with enzyme that leads to the formation of adduct. These adduct then serve as immune targets which may migrate towards the surface of the hepatocyte where they can induce the formation of antibodies, leading to inflammation and neutrophil-

mediated hepatotoxicity. This further could lead to programmed cell death (apoptosis) with immune mediated injury destroying hepatocytes by way of tumor necrosis factor (TNF) and FAS pathways[19].

The reason of decreased count of erythrocytes with the elevation in the levels of serum tranaminases and bilirubin could also be due to the development of acute immune hemolytic anemia as reported by[20]. The drug development antibody can react with the R.B.C'$_s$ leading to hemolysis. Another finding by[21], shows that there may be the development of IgM antibody that react strongly with the R.B.C'$_s$. This antibody was developed by the metabolite of diclofenac metabolism i.e. 4-hydroxy diclofenac. This could also support our finding that possibly the formation of hydroxyl diclofenac has lead to the agglutination of R.B.C'$_s$ in the blood of the rabbit, that has elevated further the level of bilirubin and was the major cause of decline in R.B.C'$_s$ count due to mediated hemolysis.

The hepatotoxic drug reactions involve moderate to severe injury to hepatocyte and is indicated by a clinical picture that resembles viral hepatitis[19]. This is characterized by a rapid onset of malaise and jaundice in association with elevated aminotranferase level which may be at least 5 times as high as normal. This is consistent with our findings indicating the rise in the level of tranaminases was very significant and was indicative of liver toxicity. The rise in liver transaminases is so high that probably if the drug was not stopped that death could have been reported. This is also true because in the previous reports and investigations on diclofenac clearly indicate that the drug should be discontinued if the symptoms are to be reversed otherwise the toxicity may be further enhanced, and become fatal.

The finding also show that diclofenac sodium is more toxic as compared to piroxicam, since the level of transaminases were increased by diclofenac sodium even often 10 days of treatment, whereas piroxicam produces significant toxicity after 30 days of treatment. Piroxicam study indicates that there may be transient elevation of SGOT and SGPT in clinical trials of patients. The study also showed elevated levels of bilirubin, jaundice, hepatitis and liver necrosis. These studies are also in accordance to our results, but are also suggestive that the Oxicams may also be hepatotoxic but only if given for long duration and in high doses. This is also confirmed by the literature which indicates that Piroxicam should be used in the lowest effective dose for the shortest possible duration.

The NSAID'$_s$ diclofenac sodium was used in both therapeutic and high doses where as Piroxicam was only used in therapeutic doses, but since it was given over 21 days, that has lead to the appearance of toxic adverse effects.

The effect of Piroxicam on hemolysis and decline in Hb and Erythrocyte counts are also in relation to our findings since Piroxicam can produce anemia and it is required that Hb and hematocrit should be routinely checked[13].

Piroxicam can elevate the bleeding time and should be given with caution if patient is maintained on warfarin. This is also related to our finding since hepatotoxicity could lead to less formation of procoagulants. This effect could also elevate bleeding time and the combination of Piroxicam with aspirin and other platelet aggregators should be avoided or used with caution[22].. Piroxicam could lead to hepatitis with cholestasis and jaundice. The liver function and jaundice was resolved after discontinuation of Piroxicam, hence Piroxicam use also leads to hemolysis leading to jaundice and abnormal liver function indicated by elevated SGOT, and SGPT. The incidence of liver toxicity by Piroxicam is also affected by the dose, which is supported by the literature indicating that in patients with hepatic insufficiency the dose needs to be adjusted.

Recent in vitro animal studies have gone some way towards demonstrating the mechanisms of NSAID induced hepatotoxicity but further work is required to fully understand the pathogenesis. Currently there are no markers and tests neither to identify those at risk of NSAID-induced hepatotoxicity, nor to identify those likely to develop hepatic failure as opposed to deranged liver function tests. While hepatotoxicity related to NSAIDS is an uncommon adverse effect, it is important to be vigilant to the hepatotoxic potential of any NSAID, as increased awareness, surveillance and reporting of these events will lead to a better understanding of the risk factors and the pathophysiology of NSAID-related hepatotoxicity. This work further can be expanded to check the effect of diclofenac & piroxicam on cardiac enzymes and kidney associated enzymes to evaluate the different toxicity profile on vital organs, and we can also see the other hematological parameters and metabolic pathways like Carbohydrate and lipid metabolism for further investigations.

CONCLUSION

In summary diclofenac sodium and piroxicam both were found to have strong anti-inflammatory agents but piroxicam has lower hepatotoxic effect after prolong use rather diclofenac sodium.the lesser toxicity profile due to its long half life, The prolonged half-life (50 hours) results in the maintenance of relatively stable plasma concentrations throughout the day on once daily doses and to significant accumulation upon multiple dosing. Also the biotransformation products of Piroxicam metabolism are reported to not have any

anti-inflammatory activity. These finding suggest that piroxicam could be a clinically useful NSAID in chronic inflammatory diseases when taken in long term use.

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